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Adnan Muslimovic

**APPLICATION FOR
UNITED STATES LETTERS PATENT**

SPECIFICATION
1543.004/12169US04

TO ALL WHOM IT MAY CONCERN:

Be it known that I, Martha Jo Whitehouse (a/k/a M.J. Whitehouse), a
citizen of the United States, residing in the State of California, have invented a new
and useful "ANGIOGENICALLY EFFECTIVE UNIT DOSE OF FGF-2 AND METHOD OF
USE" of which the following is a specification.

09385144-062799
662230-47555US

New Patent Application for: Martha Jo Whitehouse (a/k/a M.J. Whitehouse)

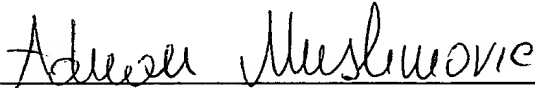
For: "ANGIOGENICALLY EFFECTIVE UNIT DOSE OF FGF-2
AND METHOD OF USE"

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Adnan Muslimovic

09385144-082799

**ANGIOGENICALLY EFFECTIVE UNIT DOSE OF FGF-2 AND
METHOD OF USE**

BACKGROUND OF THE INVENTION

A. Field of the Invention

5 The present invention is directed to a unit dose composition for inducing cardiac angiogenesis in a human comprising an therapeutically effective amount FGF-2 or an angiogenically active fragment or mutein thereof. The present invention is also directed to a method for administering a single unit dose composition to a human to induce cardiac angiogenesis while minimizing systemic risk to the patient. The present invention is useful because the disclosed unit dose composition, and method for its administration, provide an
10 alternative to angioplasty or surgical intervention for the treatment of coronary artery disease (CAD) and further provide an adjunct for reducing post myocardial infarct (MI) injury in humans.

B. Background of the Invention

15 The fibroblast growth factors (FGF) are a family of at least eighteen structurally related polypeptides (named FGF-1 to FGF-18) that are characterized by a high degree of affinity for proteoglycans, such as heparin. The various FGF molecules range in size from 15-23 kD, and exhibit a broad
20 range of biological activities in normal and malignant conditions including nerve cell adhesion and differentiation [Schubert *et al.*, *J. Cell Biol.* **104**:635-643 (1987)]; wound healing [U.S. Patent 5,439,818 (Fiddes)]; as mitogens toward many mesodermal and ectodermal cell types, as trophic factors, as differentiation inducing or inhibiting factors [Clements, *et al.*, *Oncogene* **8**:1311-1316 (1993)];
25 and as an angiogenic factor [Harada, *J. Clin. Invest.*, **94**:623-630 (1994)]. Thus,

the FGF family is a family of pluripotent growth factors that stimulate to varying extents fibroblasts, smooth muscle cells, epithelial cells and neuronal cells.

When FGF is released by normal tissues, such as in fetal development or wound healing, it is subject to temporal and spatial controls.

- 5 However, many of the members of the FGF family are also oncogenes. Thus, in the absence of temporal and spatial controls, they have the potential to stimulate tumor growth by providing angiogenesis.

- 10 Coronary artery disease is a progressive condition in humans wherein one or more coronary arteries gradually become occluded through the buildup of plaque (atherosclerosis). The coronary arteries of patients having this disease are often treated by balloon angioplasty or the insertion of stents to prop open the partially occluded arteries. Ultimately, many of these patients are required to undergo coronary artery bypass surgery at great expense and risk. It would be desirable to provide such patients with a medicament that would
15 enhance coronary blood flow so as to reduce the need to undergo bypass surgery.

- An even more critical situation arises in humans when a patient suffers a myocardial infarction, wherein one or more coronary arteries or arterioles becomes completely occluded, such as by a clot. There is an immediate need to regain circulation to the portion of the myocardium served by
20 the occluded artery or arteriole. If the lost coronary circulation is restored within hours of the onset of the infarction, much of the damage to the myocardium that is downstream from the occlusion can be prevented. The clot-dissolving drugs, such as tissue plasminogen activator (tPA), streptokinase, and urokinase, have been proven to be useful in this instance. However, as an
25 adjunct to the clot dissolving drugs, it would also be desirable to also obtain collateral circulation to the damaged or occluded myocardium by angiogenesis.

Accordingly, it is an object of the present invention to provide a medicament and a mode of administration that provides human patients with cardiac angiogenesis during coronary artery disease and/or post acute myocardial

infarction. More particularly, it is a further object of the present invention to provide a therapeutic dose of an FGF and a mode of administration to humans that provide the desired property of cardiac angiogenesis, while minimizing adverse effects.

5 Many of the various FGF molecules have been isolated and administered to various animal models of myocardial ischemia with varying and often times opposite results. According to Battler *et al.*, "the canine model of myocardial ischemia has been criticized because of the abundance of naturally occurring collateral circulation, as opposed to the porcine model, which 'excels'

10 in its relative paucity of natural collateral circulation and its resemblance to the human coronary circulation." Battler *et al.*, "*Intracoronary Injection of Basic Fibroblast Growth Factor Enhances Angiogenesis in Infarcted Swine Myocardium*," *JACC*, 22(7): 2001-6 (Dec. 1993) at page 2002, col.1. However, Battler *et al.*, who administered bovine bFGF (*i.e.*, FGF-2) to pigs in a

15 myocardial infarct model, considered the varying results that are obtained from one animal species to another, and expressly discloses that the divergent results "thus emphasiz[e] the caution that must be exercised in extrapolating results from different animal models." Battler *et al.*, at page 2005, col.1. Further, Battler points out that "the dosage and mode of administration of bFGF [*i.e.*, bovine

20 FGF-2] may have profound implications for the biologic effect achieved." Battler, *et al.*, at page 2005, col.1. Thus, it is a further object of this invention to discover a dosage and a mode of administration of a fibroblast growth factor that would provide for the safe and efficacious treatment of CAD and/or post MI injury in a human patient. More generally, it is an object of the present

25 invention to provide a pharmaceutical composition and method for inducing angiogenesis in a human heart.

SUMMARY OF THE INVENTION

664290-4T59260

The Applicants have discovered that administering a single unit dose of about 0.2 $\mu\text{g/kg}$ to about 48 $\mu\text{g/kg}$ of rFGF-2 or an angiogenically active fragment or mutein thereof into one or more coronary vessels (IC) or a peripheral vein (IV) of a human patient in need of coronary angiogenesis, unexpectedly provided the human patient with a rapid and therapeutic coronary angiogenesis that resulted in an unexpectedly large increase (*i.e.*, 96 and 100 seconds of increase in the mean change from baseline for all groups at 2 and 6 months) in the treated patient's exercise tolerance time (ETT) that persisted for an unexpectedly long duration (*i.e.*, 6 months as of this writing). These changes should result in a decreased need for standard revascularization procedures. By the term "coronary angiogenesis," as used herein, is meant the formation of new blood vessels, ranging in size from capillaries to arterioles which act as collaterals in coronary circulation. By way of comparison, angioplasty is considered a therapeutic success if it provides an increase in a patient's ETT of greater than 30 seconds compared to the placebo.

Accordingly, in one aspect, the invention is directed to a unit dose of rFGF-2 comprising a safe and therapeutically effective amount of rFGF-2 or an angiogenically active fragment or mutein thereof. Typically, the safe and therapeutically effective amount comprises about 0.2 $\mu\text{g/kg}$ to about 48 $\mu\text{g/kg}$ of rFGF-2 or an angiogenically active fragment or mutein thereof, based upon ideal body weight. In other embodiments, the safe and therapeutically effective amount of the unit dose comprises 0.2 $\mu\text{g/kg}$ to 2.0 $\mu\text{g/kg}$, greater than 2.0 $\mu\text{g/kg}$ to less than 24 $\mu\text{g/kg}$, or 24 $\mu\text{g/kg}$ to 48 $\mu\text{g/kg}$ IC of rFGF-2 or an angiogenically active fragment or mutein thereof. In another embodiment, the safe and therapeutically effective amount of the unit dose comprises 18 $\mu\text{g/kg}$ to 36 $\mu\text{g/kg}$ IV of rFGF-2 or an angiogenically active fragment or mutein thereof. Expressed in absolute terms, the unit dose of the present invention comprises .008 mg to 7.2 mg, more typically 0.3 mg to 3.5 mg, of FGF-2 or an

angiogenically active fragment or mutein thereof. A suitable FGF-2 is the rFGF-2 of SEQ ID NO: 2 or an angiogenically active fragment or mutein thereof.

In another aspect, the present invention is directed to a method of treating a human patient for CAD or to induce coronary angiogenesis therein.

- 5 The method comprises administering into one or more coronary vessels or a peripheral vein of a human patient in need of treatment for coronary artery disease (or in need of angiogenesis) a safe and therapeutically effective amount of a recombinant FGF-2 (rFGF-2) or an angiogenically active fragment or mutein thereof. Typically, a portion of the safe and therapeutically effective amount is
- 10 administered to each of two coronary vessels. The safe and therapeutically effective amount comprises about 0.2 µg/kg to about 48 µg/kg, of rFGF-2 or an angiogenically active fragment or mutein thereof in a pharmaceutically acceptable carrier. In other embodiments, the safe and therapeutically effective amount comprises 0.2 µg/kg to 2 µg/kg, >2 µg/kg to <24 µg/kg, or 24 µg/kg to 48
- 15 µg/kg of rFGF-2 an angiogenically active fragment or mutein thereof in a pharmaceutically acceptable carrier. In absolute terms, the amount of rFGF-2 or angiogenically active fragment or mutein thereof that is used in the above method comprises .008 mg to 7.2 mg, more typically 0.3 mg to 3.5 mg, of rFGF-2 or an angiogenically active fragment or mutein thereof.

- 20 Because FGF-2 is a glycosaminoglycan (*e.g.*, heparin) binding protein and the presence of a glycosaminoglycan optimizes activity and AUC (see Figs. 3 and 4), the IC dosages of RFGF-2 of the present invention typically are administered from 0-30 minutes prior to the administration of a glycosaminoglycan, such as a heparin. The heparin is administered IC or IV,
- 25 typically IV. Optionally, the heparin is combined with the unit dose composition.

Because rFGF-2 releases nitric oxide which is a potent vasodilator, aggressive fluid management prior to (proactively) and during the infusion is critical to patient's safety. Administration of IV fluids (*e.g.*, 500-1000 mL of normal saline) to establish a wedge pressure of 12 mm Hg prior to

infusion and administration of boluses of IV fluids (e.g., 200 mL normal saline) for decreases of systolic blood pressure (e.g., <90 mm Hg) associated with infusion optimized the safety of administration of rFGF-2 by IC or IV infusion to human patients.

5 Because EDTA is a potent chelator of calcium which is required for normal myocardial contraction and cardiac conduction, minimizing the concentration of EDTA is critical to patient's safety. A concentration of EDTA less than 100 µg/ml in the unit dose composition optimized the safety of administration of rFGF-2 by IC or IV infusion to human patients.

10 Because a sudden bolus of rFGF-2 is associated with profound hypotension in animals, the rate of infusion is critical to patient's safety. Administration at 0.5 to 2 mL per minute, typically 1 mL per minute, optimized the safety of administration of rFGF-2 by IC or IV infusion to human patients.

15 The unexpected magnitude and duration of the therapeutic benefit that was provided to human patients in need of coronary angiogenesis by the unit dose composition and method of administration was seen as early as two weeks after the single unit dose was administered, and persisted for 6 months after the single unit dose was administered IC or IV, as determined by measuring art-recognized clinical endpoints such as ETT, the "Seattle Angina Questionnaire"

20 (SAQ) and MRI of the target areas of the heart. In particular, when the ETT of 58 human CAD patients was assessed by treadmill at baseline, and at 1 month, 2 months, and 6 months after administration of a single unit dose of rFGF-2 by IC or IV routes, clinical benefit was observed in some patients in all dosage groups. See Table 1. Increases in exercise capacity appear between 1 and 2 months.

25 The mean ETT increased to greater than 60 seconds at 2 and 6 months with greater benefit being seen in the higher dose group (24-48 µg/kg) than in the mid (6-12 µg/kg) or low (0.33-2.0 µg/kg) dose groups. (See Table 1.) Particularly unexpected and unpredicted by animal models, were the mean increases in ETT in human patients of 93.4 and 87.5 seconds that were observed at 2 and 6

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months, respectively, post-dosing for those patients administered a unit dose of rFGF-2 by IV. Even assuming a placebo effect, the mean change from baseline for the ETT seconds still allowed an unexpectedly favorable comparison of results with angioplasty.

5 When the quality of life of 48 human CAD patients were assessed
by a validated, disease specific questionnaire, the Seattle Angina Questionnaire
(SAQ), at baseline (*i.e.*, prior to dosing), and at 2 and 6 months after a single
receiving a single unit dose of rFGF-2 of the present invention by IC or IV
routes, the mean change from baseline for the 5 scales measured by the SAQ
10 increased in a clinically significant manner for all dosage ranges whether
administered IC or IV. (Tables 2-6). In particular, the five scales assessed by
the SAQ are exertional capacity, angina stability, angina frequency, treatment
satisfaction, and disease perception. Relative to the baseline, the mean score for
exertional capacity increased by 10.9 to 20.2 at 2 months; and by 16.5 to 24.1 at
15 6 months. For angina stability, the mean score increased by 32.1 to 46.2 at 2
months; and by 16.7 to 23.2 at 6 months. For angina frequency, the mean score
increased by 20.0 to 32.9 at 2 months; and by 11.4 to 36.7 at 6 months. For
treatment satisfaction, the mean score increased by 8.5 to 19.8 at 2 months; and
by 6.3 to 19.8 at 6 months. For disease perception, the mean score increased by
20 20.2 to 27.8 at 2 months; and by 23.8 to 34.0 at 6 months. Generally, a change
of 8 points on any scale is considered clinically significant. Thus, the observed
changes of 8.5-46.2 are clinically significant for each of the five scales that were
assessed. Even assuming a placebo effect whereby a mean change from baseline
of 14 points is considered clinically significant, the results still provide for an
25 unexpectedly superior effect at almost all scales that were assessed.

As part of this study, MRI was also performed on 33 human patients diagnosed with CAD to assess the effect of administering a single unit dose of rFGF-2 on their cardiac ejection fraction, regional myocardial function and perfusion (delayed arrival zone). Specifically, the patients were

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administered a single unit dose of 0.33 µg/kg to 48 µg/kg IC or 18 µg/kg to 36 µg/kg IV of rFGF-2 of SEQ ID NO: 2. When the 33 human CAD patients were assessed by resting cardiac magnetic resonance imaging (MRI) at baseline (*i.e.*, prior to treatment), and 1, 2 and 6 months after treatment with a single unit dose of rFGF-2 of the invention by IC or IV routes, the patients exhibited a highly statistically significant response to the method of treatment as objectively measured by increased target wall thickening, target wall motion, and target area collateral extent, and by decreased target area delayed arrival extent. (Table 7) By way of summary, at 1, 2 and 6 months, the target wall thickening increased relative to baseline at 4.4%, 6.3% and 7.7%, respectively; the target wall motion increased relative to baseline at 2.7%, 4.4% and 6.4%, respectively; the target area collateral extent increased relative to baseline at 8.3%, 10.9% and 11.2%, respectively; and the target area delayed arrival extent decreased relative to baseline at -10.0%, -8.3% and -10.0%, respectively.

The above data demonstrates the clinical efficacy in humans of the present unit dose composition of rFGF-2 or an angiogenically active fragment thereof when administered IC or IV in accordance with the present invention.

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BRIEF DESCRIPTION OF THE FIGURES

Figure 1A is a plot of the mean rFGF-2 plasma concentration versus time profiles for eight different doses of rFGF-2 (SEQ ID NO: 2) administered by IC infusion in humans over a 20 minute period. The eight doses of rFGF-2 presented in Figure 1A are 0.33, 0.65, 2, 6, 12, 24, 36, and 48 $\mu\text{g/kg}$ of lean body mass (LBM).

Figure 1B is a plot of the mean FGF-2 plasma concentration versus time profiles for 2 different doses of rFGF-2 (SEQ ID NO: 2) administered by IV infusion in humans over a 20 minute period. The two IV doses of rFGF-2 in Figure 1B are 18 and 36 $\mu\text{g/kg}$. The mean concentration-time profile following IC administration of 36 $\mu\text{g/kg}$ rFGF-2 is included for comparison.

Figure 2 is a plot of mean FGF-2 area under the curve (AUC) in $\text{pg}\cdot\text{min/ml}$ corresponding to Figures 1A and 1B. This plot shows the dose linearity of systemic rFGF-2 exposure following IC or IV infusion. The systemic exposure for the IC route is similar to that observed following IV administration.

Figure 3 is a plot of individual human patient FGF-2 plasma clearance (CL) values as a function of the time of heparin administration in "minutes prior to rFGF-2 infusion" and shows the influence of timing of heparin administration on rFGF-2 plasma clearance (CL).

Figure 4 is a plot individual human patient FGF-2 dose normalized area under curves (AUCs) as a function of the time of heparin administration in "minutes prior to rFGF-2 infusion" and shows the influence of timing of heparin administration on FGF-2 AUC.

DETAILED DESCRIPTION OF THE INVENTION

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The Applicants have discovered that a single dose of rFGF-2 or an angiogenically active fragment or mutein thereof, when administered in a safe and therapeutically effective amount into one or more coronary vessels or into a peripheral vein of a human patient diagnosed with CAD provides the patient with a safe and therapeutically efficacious treatment for the patient's coronary artery disease that lasts at least 4 to 6 months, more typically at least six months, before a further treatment is needed. This duration of the effect and the magnitude of the improvements in ETT, SAQ and MRI were unexpected for a single unit dose of medicament.

By the phrase "therapeutically effective amount" or "safe and therapeutically effective amount" as used herein in relation to rFGF-2 is meant an amount of rFGF-2 or an angiogenically active fragment or mutein thereof that when administered in accordance with this invention, is free from major complications that cannot be medically managed, and that provides for objective cardiac improvement in patients having symptoms of CAD despite optimum medical management. Thus, acute hypotension that can be managed by administration of fluids, is considered "safe" for the purpose of this invention. Typically, the safe and therapeutically effective amount of rFGF-2 comprises about 0.2 µg/kg to about 48 µg/kg of rFGF-2 or an angiogenically active fragment or mutein thereof. A suitable FGF-2 for use in the present invention is the rFGF-2 of SEQ ID NO: 2 or an angiogenically active fragment or mutein thereof.

Accordingly, the present invention has multiple aspects. In its first aspect, the present invention is directed to a unit dose composition for inducing angiogenesis in a human patient, the unit dose comprising a therapeutically effective (*i.e.*, an angiogenically effective) amount of rFGF-2 or an angiogenically active fragment or mutein thereof, the amount comprising

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about 0.2 µg/kg to about 48 µg/kg of rFGF-2 or an angiogenically active fragment or mutein thereof.

By the term "unit dose composition" as used herein is meant a composition that when administered to a human patient in accordance with the method of the present invention provides a typical human patient in need of angiogenesis with an angiogenic effect of significant efficacy so as not to require retreatment for at least 4-6 months, typically 6 months. The unit dose composition of the present invention is typically provided in combination with one or more pharmaceutically acceptable excipients or carriers. In other embodiments of the unit dose composition, a safe and therapeutically effective amount comprises about 0.2 µg/kg to about 2 µg/kg, about 2 µg/kg to about 24 µg/kg, or about 24 µg/kg to about 48 µg/kg of rFGF-2 or an angiogenically active fragment or mutein thereof.

It is convenient to define the unit dose composition of the present invention in more absolute terms that are not dependent upon the weight of the patient to be treated. When so defined, the unit dose composition comprises from .008 mg to 7.2 mg of rFGF-2 or an angiogenically active fragment or mutein thereof. In this embodiment, the unit dose composition contains a sufficient amount of FGF-2 to accommodate dosing any one of the majority of human CAD patients, ranging from the smallest patient (*e.g.*, 40 kg) at the lowest dosage (about 0.2 µg/kg) through the larger patients (*e.g.*, 150 kg) at the highest dosage (about 48 µg/kg). More typically, the unit dose comprises 0.3 mg to 3.5 mg of rFGF-2 or an angiogenically active fragment or mutein thereof. The unit dose composition is typically provided in solution or lyophilized form containing the above referenced amount of rFGF-2 and an effective amount of one or more pharmaceutically acceptable buffers, stabilizers and/or other excipients as later described herein.

The active agent in the Applicants' above described unit dose composition is a recombinant FGF-2 or an angiogenically active fragment or

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mutein thereof. Methods for making recombinant FGF-2 are well-known in the art. The recombinant FGF-2 of SEQ ID NO: 2 is made as described in U.S. Patent 5,155,214, entitled "Basic Fibroblast Growth Factor," which issued on October 13, 1992, and which is expressly incorporated herein by reference in its entirety. Moreover, all other references cited herein, whether occurring before or after this sentence, are expressly incorporated herein by reference in their entirety. As disclosed in the '214 patent, a DNA of SEQ ID NO: 1, which encodes a bFGF (hereinafter "FGF-2") of SEQ ID NO: 2, is inserted into a cloning vector, such as pBR322, pMB9, Col E1, pCRI, RP4 or λ -phage, and the cloning vector is used to transform either a eukaryotic or prokaryotic cell, wherein the transformed cell expresses the FGF-2. In one embodiment, the host cell is a yeast cell, such as *Saccharomyces cerevisiae*. The resulting full length FGF-2 that is expressed has 146 amino acids in accordance with SEQ ID NO: 2. Although the FGF-2 of SEQ ID NO: 2 has four cysteines, *i.e.*, at residue positions 25, 69, 87 and 92, there are no internal disulfide linkages. ['214 at col. 6, lines 59-61.] However, in the event that cross-linking occurred under oxidative conditions, it would likely occur between the residues at positions 25 and 69.

The FGF-2 of SEQ ID NO: 2, which has 146 amino acid residues, differs from naturally occurring human FGF-2 by only two amino acid residue. In particular, the amino acids at residue positions 112 and 128 of the FGF-2 of SEQ ID NO: 2 are Ser and Pro, respectively, whereas in human FGF-2, they are Thr and Ser, respectively. In nature, bovine FGF-2, like the corresponding human FGF-2 is initially synthesized *in vivo* as a polypeptide having 155 amino acid residues. Abraham *et al.* "Human Basic Fibroblast Growth Factor: Nucleotide Sequence and Genomic Organization," *EMBO J.*, 5(10):2523-2528 (1986). When the FGF-2 of SEQ ID NO: 2 is compared to the full length 155 residue bovine FGF-2 of Abraham, the FGF-2 of SEQ ID NO: 2 lacks the first nine amino acid residues, Met Ala Ala Gly Ser Ile Thr Thr Leu (SEQ ID NO:

3), at the N-terminus of the corresponding full length molecule. The recombinant FGF-2 employed in the present compositions and method was purified to pharmaceutical quality (98% or greater purity) using the techniques described in detail in U.S. Pat. 4,956,455, entitled "Bovine Fibroblast Growth Factor" which issued on 09/11/90 and which is incorporated herein by reference in its entirety. In particular, the first two steps employed in the purification of the recombinant FGF-2 of Applicants' unit dose composition are "conventional ion-exchange and reverse phase HPLC purification steps as described previously." [U.S. Pat. 4,956,455, citing to Bolen *et al.*, PNAS USA 81:5364-5368 (1984).] The third step, which the '455 patent refers to as the "key purification step" ['455 at col. 7, lines 5-6], is heparin-SEPHAROSE® affinity chromatography, wherein the strong heparin binding affinity of the FGF-2 is utilized to achieve several thousand-fold purification when eluting at approximately 1.4M and 1.95M NaCl ['455 at col. 9, lines 20-25]. Polypeptide homogeneity was confirmed by reverse-phase high pressure liquid chromatography (RP-HPLC). Buffer exchange was achieved by SEPHADEX® G-25(M) gel filtration chromatography.

In addition to the 146 residue rFGF-2 of SEQ ID NO: 2, the active agent in the unit dose of the present invention also comprises an "angiogenically active fragment" of FGF-2. By the term "angiogenically active fragment" of FGF-2 is meant a fragment of FGF-2 that has about 80% of the 146 residues of SEQ ID NO: 2 and that retains at least 50%, preferably at least 80%, of the angiogenic activity of the FGF-2 of SEQ ID NO: 2.

To be angiogenically active, the FGF-2 fragment should have two cell binding sites and at least one of the two heparin binding sites. The two putative cell binding sites of the analogous human FGF-2 occur at residue positions 36-39 and 77-81 thereof. See Yoshida, *et al.*, "Genomic Sequence of *hst*, a Transforming Gene Encoding a Protein Homologous to Fibroblast Growth Factors and the *int-2*-Encoded Protein," PNAS USA, 84:7305-7309 (Oct. 1987)

(4)

at Fig. 3. The two putative heparin binding sites of hFGF-2 occur at residue positions 18-22 and 107-111 thereof. See Yoshida (1987) at Fig. 3. Given the greater than 98% similarity between the amino acid sequences for naturally occurring human FGF-2 (hFGF-2) and rFGF-2 (SEQ ID NO: 2), it is expected that the two cell binding sites for rFGF-2 (SEQ ID NO: 2) are also at residue positions 36-39 and 77-81 thereof, and that the two heparin binding sites are at residue positions 18-22 and 107-111 thereof. Consistent with the above, it is well known in the art that N-terminal truncations of the FGF-2 of SEQ ID NO: 2 do not eliminate its activity in cows. In particular, the art discloses several naturally occurring and biologically active fragments of the FGF-2 that have N-terminal truncations relative to the FGF-2 of SEQ ID NO: 2. An active and truncated bFGF-2 having residues 12-146 of SEQ ID NO: 2 was found in bovine liver and another active and truncated bFGF-2, having residues 16-146 of SEQ ID NO: 2 was found in the bovine kidney, adrenal glands and testes. [See U.S. Pat. 5,155,214 at col. 6, lines 41-46, citing to Ueno, *et al.*, Biochem and Biophys Res. Comm., 138:580-588 (1986).] Likewise, other fragments of the bFGF-2 of SEQ ID NO: 2 that are known to have FGF activity are FGF-2 (24-120)-OH and FGF-2 (30-110)-NH₂. [U.S. Pat. 5,155,214 at col. 6, lines 48-52.] These latter fragments retain both of the cell binding portions of FGF-2 (SEQ ID NO: 2) and one of the heparin binding segments (residues 107-111). Accordingly, the angiogenically active fragments of FGF-2 typically encompass those terminally truncated fragments of FGF-2 that have at least residues that correspond to residues 30-110 of FGF-2 of SEQ ID NO: 2; more typically, at least residues that correspond to residues 18-146 of FGF-2 of SEQ ID NO: 2.

The unit dose of the present invention also comprises an "angiogenically active . . . mutein" of the rFGF-2 of SEQ ID NO: 2. By the term "angiogenically active . . . mutein" as used herein, is meant an isolated and purified recombinant protein or polypeptide that has 65% sequence identity (homology) to any naturally occurring FGF-2, as determined by the Smith-

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Waterman homology search algorithm (*Meth. Mol. Biol.* 70:173-187 (1997)) as implemented in MSPRCH program (Oxford Molecular) using an affine gap search with the following search parameters: gap open penalty of 12, and gap extension penalty of 1, and that retains at least 50%, preferably at least 80%, of the angiogenic activity of the naturally occurring FGF-2 with which it has said at least 65% sequence identity. Preferably, the angiogenically active mutein has at least 75%, more preferably at least 85%, and most preferably, at least 90% sequence identity to the naturally occurring FGF-2. Other well-known and routinely used homology/identity scanning algorithm programs include Pearson and Lipman, *PNAS USA*, 85:2444-2448 (1988); Lipman and Pearson, *Science*, 222:1435 (1985); Devereaux *et al.*, *Nuc. Acids Res.*, 12:387-395 (1984); or the BLASTP, BLASTN or BLASTX algorithms of Altschul, *et al.*, *Mol. Biol.*, 215:403-410 (1990). Computerized programs using these algorithms are also available and include, but are not limited to: GAP, BESTFIT, BLAST, FASTA and TFASTA, which are commercially available from the Genetics Computing Group (GCG) package, Version 8, Madison WI, USA; and CLUSTAL in the PC/Gene program by Intellegentics, Mountain View CA. Preferably, the percentage of sequence identity is determined by using the default parameters determined by the program.

The phrase "sequence identity," as used herein, is intended to refer to the percentage of the same amino acids that are found similarly positioned within the mutein sequence when a specified, contiguous segment of the amino acid sequence of the mutein is aligned and compared to the amino acid sequence of the naturally occurring FGF-2.

When considering the percentage of amino acid sequence identity in the mutein, some amino acid residue positions may differ from the reference protein as a result of conservative amino acid substitutions, which do not affect the properties of the protein or protein function. In these instances, the percentage of sequence identity may be adjusted upwards to account for the

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similarity in conservatively substituted amino acids. Such adjustments are well-known in the art. See, e.g., Meyers and Miller, "Computer Applic. Bio. Sci., 4:11-17 (1988).

To prepare an "angiogenically active mutein" of an angiogenic agent of the present invention, one uses standard techniques for site directed mutagenesis, as known in the art and/or as taught in Gilman, *et al.*, *Gene*, 8:81 (1979) or Roberts, *et al.*, *Nature*, 328:731 (1987). Using one of the site directed mutagenesis techniques, one or more point mutations are introduced into the cDNA sequence of SEQ ID NO: 1 to introduce one or more amino acid substitutions or an internal deletion. Conservative amino acid substitutions are those that preserve the general charge, hydrophobicity/hydrophilicity, and/or steric bulk of the amino acid being substituted. By way of example, substitutions between the following groups are conservative: Gly/Ala, Val/Ile/Leu, Lys/Arg, Asn/Gln, Glu/Asp, Ser/Cys/Thr, and Phe/Trp/Tyr. Significant (up to 35%) variation from the sequence of the naturally occurring angiogenic FGF-2 is permitted as long as the resulting protein or polypeptide retains angiogenic activity within the limits specified above.

Cysteine-depleted muteins are muteins within the scope of the present invention. These muteins are constructed using site directed mutagenesis as described above, or according to the method described in U.S. Pat. 4,959,314 ("the `314 patent"), entitled "Cysteine-Depleted Muteins of Biologically Active Proteins." The `314 patent discloses how to determine biological activity and the effect of the substitution. Cysteine substitution is particularly useful in proteins having two or more cysteines that are not involved in disulfide formation. Suitable substitutions include the substitution of serine for one or both of the cysteines at residue positions 87 and 92, which are not involved in disulfide formation. Preferably, substitutions are introduced at the FGF-2 N-terminus, which is not associated with angiogenic activity. However, as

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discussed above, conservative substitutions are suitable for introduction throughout the molecule.

5 The unit dose composition of the present invention comprises a safe and an angiogenically effective dose of rFGF-2 or an angiogenically active fragment or mutein thereof, and a pharmaceutically acceptable carrier. Typically, the safe and angiogenically effective dose of the pharmaceutical composition of the present invention is in a form and a size suitable for administration to a human patient and comprises (i) 0.2 $\mu\text{g/kg}$ to 48 $\mu\text{g/kg}$ of rFGF-2 or an angiogenically active fragment or mutein thereof, (ii) and a pharmaceutically acceptable carrier. In other embodiments, the safe and angiogenically effective dose comprises 0.2 $\mu\text{g/kg}$ to 2 $\mu\text{g/kg}$, >2 $\mu\text{g/kg}$ to <24 $\mu\text{g/kg}$ or 24 $\mu\text{g/kg}$ to 48 $\mu\text{g/kg}$ of FGF-2 or an angiogenically active fragment or mutein thereof, and a pharmaceutically acceptable carrier. Expressed in absolute terms for the majority of human CAD patients, the unit dose of the present invention comprises .008 mg to 7.2 mg, more typically 0.3 mg to 3.5 mg, of the FGF-2 or an angiogenically active fragment or mutein thereof.

10 The second recited component of the unit dose composition of the present invention is a "pharmaceutically acceptable carrier." By the term "pharmaceutically acceptable carrier" as used herein is meant any of the carriers or diluents that are well-known in the art for the stabilization and/or administration of a proteinaceous medicament that does not itself induce the production of antibodies harmful to the patient receiving the composition, and which may be administered without undue toxicity. The choice of the pharmaceutically acceptable carrier and its subsequent processing enables the unit dose composition of the present invention to be provided in either liquid or solid form.

25 When the unit dose composition is in liquid form, the pharmaceutically acceptable carrier comprises a stable carrier or diluent suitable for intravenous ("IV") or intracoronary ("IC") injection or infusion. Suitable

carriers or diluents for injectable or infusible solutions are nontoxic to a human recipient at the dosages and concentrations employed, and include sterile water, sugar solutions, saline solutions, protein solutions or combinations thereof.

Typically, the pharmaceutically acceptable carrier includes a
5 buffer and one or more stabilizers, reducing agents, anti-oxidants and/or anti-oxidant chelating agents. The use of buffers, stabilizers, reducing agents, anti-oxidants and chelating agents in the preparation of protein based compositions, particularly pharmaceutical compositions, is well-known in the art. See, Wang
10 *et al.*, "Review of Excipients and pHs for Parenteral Products Used in the United States," *J. Parent. Drug Assn.*, **34**(6):452-462 (1980); Wang *et al.*, "Parenteral Formulations of Proteins and Peptides: Stability and Stabilizers," *J. Parent. Sci. and Tech.*, **42**:S4-S26 (Supplement 1988); Lachman, *et al.*, "Antioxidants and Chelating Agents as Stabilizers in Liquid Dosage Forms-Part 1," *Drug and Cosmetic Industry*, **102**(1): 36-38, 40 and 146-148 (1968); Akers, M.J.,
15 "Antioxidants in Pharmaceutical Products," *J. Parent. Sci. and Tech.*, **36**(5):222-228 (1988); and Methods in Enzymology, Vol. XXV, Colowick and Kaplan Eds., "Reduction of Disulfide Bonds in Proteins with Dithiothreitol," by Konigsberg, pages 185-188. Suitable buffers include acetate, adipate, benzoate, citrate, lactate, maleate, phosphate, tartarate and the salts of various amino acids.
20 See Wang (1980) at page 455. Suitable stabilizers include carbohydrates such as threlose or glycerol. Suitable reducing agents, which maintain the reduction of reduced cysteines, include dithiothreitol (DTT also known as Cleland's reagent) or dithioerythritol at 0.01% to 0.1% wt/wt; acetylcysteine or cysteine at 0.1% to 0.5% (pH 2-3); and thioglycerol at 0.1% to 0.5% (pH 3.5 to 7.0) and
25 glutathione. See Akers (1988) at pages 225 to 226. Suitable antioxidants include sodium bisulfite, sodium sulfite, sodium metabisulfite, sodium thiosulfate, sodium formaldehyde sulfoxylate, and ascorbic acid. See Akers (1988) at pages 225. Suitable chelating agents, which chelate trace metals to prevent the trace metal catalyzed oxidation of reduced cysteines, include citrate, tartarate,

ethylenediaminetetraacetic acid (EDTA) in its disodium, tetrasodium, and calcium disodium salts, and diethylenetriamine pentaacetic acid (DTPA). *See e.g.*, Wang (1980) at pages 457-458 and 460-461, and Akers (1988) at pages 224-227. Suitable sugars include glycerol, trehalose, glucose, galactose and mannitol, sorbitol. A suitable protein is human serum albumin.

In liquid form, a typical unit dose composition of the present invention comprises from about 0.001 mg to 8 mg, more typically 0.03 to 5 mg rFGF-2 or an angiogenically active fragment or mutein thereof, dissolved a pharmaceutically acceptable carrier. A suitable pharmaceutically acceptable carrier comprises 10 mM thioglycerol, 135 mM NaCl, 10 mM sodium citrate, and 1 mM EDTA, pH 5. A suitable diluent or flushing agent for the above-described unit dose composition is any of the above-described carriers. Typically, the diluent is the carrier solution. rFGF-2 or an angiogenically active fragment or mutein thereof is unstable for long periods of time in liquid form. To maximize stability and shelf life of the liquid form, the unit dose composition should be stored frozen at -60°C. When thawed, the solution is stable for 6 months at refrigerated conditions. A typical unit dose would comprise about 1-40 ml, more typically 10-40 ml, of the above-described composition having 0.008-7.2 mg of rFGF-2 or an angiogenically active fragment or mutein dissolved therein. A suitable rFGF-2 for use in the unit dose is the rFGF-2 of SEQ ID NO: 2 or an angiogenically active fragment or mutein thereof.

In another embodiment, the unit dose composition is provided in lyophilized (freeze-dried) form. In this form, the unit dose of rFGF-2 is capable of being stored at refrigerated temperatures for substantially longer than 6 months without loss of therapeutic effectiveness. Lyophilization is accomplished by the rapid freeze drying (*i.e.*, removing water) under reduced pressure of a plurality of vials, each containing the above described liquid form of the unit dose of the rFGF-2 of the present invention therein. Lyophilizers, which perform the above described lyophilization, are commercially available and

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readily operable by those skilled in the art. The resulting lyophilized unit dose composition, in lyophilized cake form, is formulated to contain within the resulting lyophilized cake one or more of the buffers, stabilizers, anti-oxidants, reducing agents, salts and/or sugars described above for the corresponding liquid
5 formulation. A lyophilized unit dose composition containing all such other components need only be reconstituted to a known volume or concentration with sterile aqueous diluent such as sterile water, a sterile sugar solution, or a sterile saline solution. Alternatively, it could be reconstituted with a sterile buffer solution as described above, but lacking a chelating agent, such as EDTA. As a
10 lyophilized cake, the unit dose composition is stable from 6 months to two years at refrigerated temperatures. Thus, storage of the unit dose composition in lyophilized form is readily accommodated using conventional refrigeration equipment.

Because the unit dose composition of the present invention is
15 administered via a cardiac catheter or other injection device, which has dead space, it is convenient to formulate the vial containing the unit dose composition so that it contains about 10-50% more of the rFGF-2 or angiogenically active fragment or mutein thereof than is to be administered to the patient. For example, when the unit dose of the rFGF-2 to be administered is 7.2 mg, the vial
20 is optionally formulated to contain up to 50% extra (*e.g.*, a total of about 10.8 mg) of rFGF-2 or angiogenically active fragment or mutein thereof. The extra solution is suitable for filling the dead space in the delivery equipment. In an alternative embodiment that does not allow for dead space, the pharmaceutical composition is loaded in the cardiac catheter in front of a pharmaceutically
25 acceptable buffer, diluent or carrier, which is then used to deliver the appropriate amount of the one or more dosages to the one or more sites in the myocardium that are in need of angiogenesis.

As discussed above, the pharmaceutically acceptable carrier for the above described unit dose composition comprises a buffer and one or more

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stabilizers, reducing agents, anti-oxidants and/or anti-oxidant chelating agents. It is also within the scope of the present invention that the unit dose composition contain an amount of a glycosaminoglycan (also known as a "proteoglycan" or a "mucopolysaccharide"), such as heparin, that is effective to bind to the FGF-2 and to the endothelial cell receptors so as to enhance the angiogenic effectiveness of the FGF-2 or angiogenically active fragment or mutein thereof. The amount of heparin that is administered is about 10-80 U per kg of patient weight (U/kg), typically about 40 U/kg. Expressed in absolute terms, the total amount of heparin administered to any one patient does not exceed 5,000 U. Thus, upon reconstitution, the unit dose composition of the present invention would not only contain an angiogenically effective amount of rFGF-2 or an angiogenically active fragment or mutein thereof, it would also contain from about 10-80 U/kg of heparin, typically about 40 U/kg. The typical volume of diluent is from about 1 to 40 ml. While larger volumes of diluent could be used, such larger volumes would typically result in longer administration times. Depending upon the weight of the patient in kg, a single dose comprising from 0.2 µg/kg to 48 µg/kg of the rFGF-2 or an angiogenically active fragment or mutein thereof is withdrawn from the vial as reconstituted product for administration to the patient. Thus, an average 70 kg man that is being dosed at 24 µg/kg would have a sufficient volume of the reconstituted product withdrawn from the vial to receive an IC infusion of (70 kg x 30 µg/kg) 2100 µg (*i.e.*, 2.1 mg).

In its second aspect, the present invention is directed to a method for treating a human patient for CAD or MI, using the above described unit dose composition. In particular, in one embodiment, the present invention is directed to a method for treating a human patient for coronary artery disease, comprising administering a safe and therapeutically effective amount of a recombinant FGF-2 or an angiogenically active fragment or mutein thereof to one or more, typically two, patent coronary vessels or a peripheral vein of a human patient in need of treatment for coronary artery disease. The human patient in need of

Q treatment for coronary artery disease is typically a human patient with coronary artery disease who remains symptomatic with angina despite ^{optimal} ~~optional~~ medical management. A preferred coronary vessel is a coronary artery, although grafted saphenous veins and grafted internal mammary arteries, as provided by coronary angioplasty, are also suitable. Suitable peripheral veins for administering the unit dose composition include those peripheral veins found throughout the human body that are routinely used by treating physicians and nurses for administration of fluids and medicaments. Examples of such veins include the cephalic, the median cubital, and the basilic of the arm.

10 When administered as an intracoronary (IC) infusion, the unit dose of rFGF-2 or angiogenic fragment or mutein thereof is typically administered within an hour, more typically over a period of about 20 minutes into one or more (typically, two) patent coronary vessels. When administered over a twenty minute period, the unit dose composition is typically administered at a rate of 0.5
15 to 2.0 ml/minute, more typically at about 1 ml/minute. The coronary vessels can be native vessels or grafts, so long as they are not occluded. The volume of the unit dose of rFGF-2 or angiogenic fragment or mutein thereof is typically 10-40 ml; more typically 20 ml. The length of time for infusion of the unit dose is not critical and can be shortened or lengthened depending on the rate and volume of
20 infusion

When administered as an intravenous (IV) infusion, the unit dose of rFGF-2 or angiogenic fragment or mutein thereof is administered typically within an hour, more typically over a 20 minute period, into a peripheral vein using a conventional IV setup. When administered over a twenty minute period,
25 the unit dose composition is typically administered at a rate of 1 ml/minute.

In the phase I clinical trial of the above described method for treating CAD, a single unit dose composition was administered IC or IV to human patients having CAD who remained symptomatic with angina despite optional medical management. Because the method of the present invention induces

angiogenesis, the method of the present invention provides treatment of the underlying condition in CAD or MI and not merely transitory relief from the symptoms, such as provided by nitrates. Typically, the safe and therapeutically effective amount of the method of the present invention comprises 0.2 µg/kg to 48 µg/kg of rFGF-2 or an angiogenically active fragment or mutein thereof in a pharmaceutically acceptable carrier. In other embodiments, the safe and therapeutically effective amount comprises 0.2 µg/kg to 2 µg/kg, >2 µg/kg to <24 µg/kg, or 24 µg/kg to 48 µg/kg of rFGF-2 or an angiogenically active fragment or mutein thereof in a pharmaceutically acceptable carrier. In absolute terms, the safe and therapeutically effective amount is about .008 mg to about 7.2 mg of rFGF-2 or an angiogenically active fragment or mutein thereof; more typically, 0.3 mg to 3.5 mg of rFGF-2 or an angiogenically active fragment or mutein thereof. A suitable rFGF-2 is the rFGF-2 of SEQ ID NO: 2 or an angiogenically active fragment or mutein thereof.

In another aspect, the present invention is also directed to a method for inducing angiogenesis in a heart of a human patient comprising, administering a single unit dose composition of a recombinant FGF-2 or an angiogenically active fragment or mutein thereof to one or more coronary vessels or to a peripheral vein in a human patient in need of coronary angiogenesis, said unit dose composition comprising from about .008 mg to 7.2 mg of recombinant rFGF-2 or an angiogenically active fragment or mutein thereof in a pharmaceutically acceptable carrier. More typically, the unit dose composition comprises about 0.3-3.5 mg rFGF-2 or an angiogenically active fragment or mutein thereof in a pharmaceutically acceptable carrier. As described above, a single unit dose composition containing a therapeutically effective amount of an rFGF-2 or an angiogenically fragment or mutein thereof is administered to at least one coronary vessel of a human patient in need of angiogenesis, using standard cardiac catheterization techniques already known and used in the art for the intracoronary administration of medicaments, *e.g.*, thrombolytics,

streptokinase, or radio-opaque dyes or magnetic particles used to visualize the coronary arteries. By way of example, a coronary catheter is inserted into an artery (*e.g.*, femoral or subclavian) of the patient in need of treatment and the catheter is pushed forward, with visualization, until it is positioned in the appropriate coronary vessel of the patient to be treated. Using standard precautions for maintaining a clear line, the pharmaceutical composition in solution form is administered by infusing the unit dose substantially continuously over a period of 10 to 30 minutes. Although the pharmaceutical composition of the invention could be administered over a longer period of time, the Applicants perceive no benefit and a potentially increased risk of thrombosis in doing so. Typically, a portion (*e.g.*, one half) of the unit dose is administered in a first coronary vessel. Then, the catheter is repositioned into a second secondary coronary vessel and the remainder of the unit dose is administered with flushing of the catheter. Using the above-described repositioning procedure, portions of the unit dose may be administered to a plurality of coronary vessels until the entire unit dose has been administered. After administration, the catheter is withdrawn using conventional art known protocols. In the phase I clinical trials described herein, therapeutic benefit was reported by patients as early as two weeks following the IC rFGF-2 administration of a single unit dose. Clinically significant improvement was demonstrable by objective criterion (ETT and/or SAQ) as early as 30 days following IC or IV administration of a single unit dose of the present invention, and was maintained for six months following dosing. In certain patients with progressive CAD disease, it may be necessary or appropriate to administer additional unit doses of rFGF-2 at six or 12 month intervals after the initial unit dose, to overcome the progression of the CAD during that interim period. In some patients with very progressive CAD, unit doses of present invention would be readministered at 4 month intervals. In any instance, the treating physician would be able to determine the time, if any, for

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readministration based upon routine assessment of the clinical symptoms of the patient.

One of the benefits of the method of the present invention is cardiac angiogenesis. Accordingly, in another aspect, the present invention is directed to a method for inducing angiogenesis in a heart of a human patient, comprising administering into one or more coronary vessels (IC) or into a peripheral vein (IV) of a human patient in need of coronary angiogenesis, a single unit dose composition comprising an angiogenically effective amount of rFGF-2 or an angiogenically active fragment or mutein thereof in a pharmaceutically acceptable carrier. In the above method, the angiogenically effective amount comprises about 0.2 $\mu\text{g/kg}$ to about 48 $\mu\text{g/kg}$ (or in absolute terms about .008 mg to about 7.2 mg) of a recombinant FGF-2 or an angiogenically active fragment or mutein thereof. More typically, the angiogenically effective amount comprises about 0.3 mg to 3.5 mg of a recombinant FGF-2 or an angiogenically active fragment or mutein thereof. A suitable rFGF-2 for use in the above-identified method is the rFGF-2 of SEQ ID NO: 2 or an angiogenically active fragment thereof. In one embodiment of the above method, the unit dose composition is administered IC to patent coronary vessels or IV to a peripheral vein. In another embodiment, the unit dose composition is administered with heparin as described herein.

The above described method for providing coronary angiogenesis is also beneficial in human patients that have undergone a myocardial infarction (MI) in one or more coronary arteries. Accordingly, in another aspect, the present invention is also directed to a method for treating a human patient for an MI comprising, administering into one or more coronary vessels or into a peripheral vein of said human patient, a single unit dose composition comprising a therapeutically effective amount of rFGF-2 or an angiogenically active fragment or mutein thereof. In the above method, the unit dose composition typically comprises about 0.2 $\mu\text{g/kg}$ to about 48 $\mu\text{g/kg}$ (or in absolute terms

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about .008 mg to about 7.2 mg) of a recombinant FGF-2 or an angiogenically active fragment or mutein thereof in a pharmaceutically acceptable carrier. A suitable rFGF-2 for use in the above-identified method is the rFGF-2 of SEQ ID NO: 2 or an angiogenically active fragment thereof.

5 In the event of unstable angina or acute myocardial infarction, requiring angioplasty, the same doses of rFGF-2 or angiogenic fragment or mutein thereof that are disclosed herein would also be useful as an adjunct therapy in treating those conditions. Accordingly, in another aspect, the present invention is directed to an improved method for treating a patient for unstable
10 angina or acute myocardial infarction, requiring angioplasty, the method comprising providing angioplasty to the patient in need of treatment; the improvement comprising administering into one or more coronary vessels or into a peripheral vein of said human patient, a single unit dose composition comprising a therapeutically effective amount of rFGF-2 or an angiogenically
15 active fragment or mutein thereof. In the above method, the unit dose composition comprises about 0.2 µg/kg to about 48 µg/kg (or in absolute terms about .008 mg to about 7.2 mg) of a recombinant FGF-2 or an angiogenically active fragment or mutein thereof in a pharmaceutically acceptable carrier. A suitable rFGF-2 for use in the above-identified method is the rFGF-2 of SEQ ID
20 NO: 2 or an angiogenically active fragment thereof.

 In any of the above-described methods of the present invention, the rFGF-2 or the angiogenically active fragment or mutein thereof is associated with release of nitric oxide, a recognized smooth muscle dilator, which upon administration to the patient causes a sudden drop in the patient's blood pressure.
25 Accordingly, in the methods of the present invention, it is preferable to hydrate the patient with IV fluids prior to administering the unit dose of the present invention. Moreover, for safety and tolerability of the unit dose, aggressive fluid management during and after rFGF-2 administration is also preferred. Finally, it is also within the scope of the above described methods to include the step of

administering an effective amount of a glycosoaminoglycan (also known as a "proteoglycan" or a "mucopolysaccharide"), such as heparin from 0-30 minutes prior to administering the unit dose composition of the present invention. Typically, the effective amount of glycosaminoglycan (such as heparin) that is administered is about 10-80 U/kg, more typically, about 40 U/kg. However, the total amount of heparin administered to any one patient immediately prior to dosing generally does not exceed 5,000 U.

Because EDTA is a potent chelator of calcium which is required for normal myocardial contraction and cardiac conduction, minimizing the concentration of EDTA is critical to patient's safety. A concentration of EDTA less than 100 µg/ml optimized the safety of administration of rFGF-2 by IC or IV infusion to human patients.

Because a sudden bolus of rFGF-2 is associated with profound hypotension in animals, the rate of infusion is critical to patient's safety. Administration at 0.5 to 2 mL per minute, typically 1 mL per minute, optimized the safety of administration of rFGF-2 by IC or IV infusion to human patients.

A Phase I clinical trial directed to treating human patients for CAD by administering a single unit dose composition of the present invention was conducted and is described in Examples 1-3 herein. In that trial, sixty-six (66) human patients diagnosed with CAD, who satisfied the criteria of Example 2 herein, received a single unit dose of rFGF-2 in accordance with the method of the present invention. Specifically, fifty-two human patients were administered a unit dose of 0.33 µg/kg to 48 µg/kg of rFGF-2 by IC infusion over about a 20 minute period. Fourteen human patients were administered a unit dose of either 18 µg/kg or 36 µg/kg of rFGF-2 by IV infusion over about a 20 minute period. The 66 treated patients were then assessed relative to baseline (*i.e.*, prior to treatment with the single unit dose), and again at 1 month, 2 months and 6 months after treatment with the single unit dose, using three sets of art-recognized assessment criteria: 1) changes in their exercise tolerance time (ETT);

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2) the Seattle Angina Questionnaire, which provides an assessment based upon a mixed combination of objective and subjective criteria; and 3) the measurement of physical changes in the heart as assessed by MRI.

For ETT of the 66 patients of the Phase I clinical trial of Examples 1-3 was measured at baseline, and at 1 month, 2 months and 6 months after dosing (with a single unit dose composition of the invention) using a Bruce treadmill protocol. Subjects were excluded from the analysis if the treadmill protocol was not the same as used at baseline. Therefore, the number of subjects varied over time. In addition, any patients who had emergency revascularization were excluded from the analysis. A dose was considered effective if the mean change in ETT from baseline increased by greater than 60 seconds. The results of the ETT assessment are provided in Table 1.

Table 1. Exercise Tolerance Time (ETT) - Change from Baseline

<i>FGF-2 Dose Group</i>	<i>Change from Baseline at One Month</i>	<i>Change from Baseline at Two Months</i>	<i>Change from Baseline at Six Months</i>
0.33 to 2.0 µg/kg IC (N = 16) (low)	N = 8 45.1 sec (-105 to 180)	N = 6 130.0 sec* (19 to 240)	N = 5 60.8 sec (-45 to 210)
6.0 and 12 µg/kg IC (N = 8) (mid)	N = 2 -24.0 sec (-48 to 0)	N = 4 -2.5 sec (-90 to 120)	N = 2 6.5 sec (-0 to 13)
24.0 to 48.0 µg/kg IC (N = 28) (high)	N = 18 51.9 sec (-188 to 399)	N = 21 107.9 sec* (-30 to 385)	N = 16 133.1 sec* (-195 to 386)
18.0 & 36.0 µg/kg IV (N = 14)	N = 12 45.1 sec (-75 to 237)	N = 12 93.4 sec* (0 to 285)	N = 12 87.5 sec* (-60 to 285)
ALL GROUPS (N = 66)	N = 40 45.0 sec	N = 43 96.0 sec	N = 35 100.0 sec

N = number of subjects; mean; (range in seconds); * = p < 0.05

Referring to Table 1, the mean change from baseline at one month was less than 60 seconds for all dose groups. However, the percentage of patients stopping

their treadmill test because of angina decreased in all groups over time. At 2 months and 6 months after dosing, the mean changes from baseline were greater in the high dose IC and IV groups of patients than in the low and mid dose IC groups. The persistence of increased ETT at 6 months (133.1 sec and 87.5 sec) 5 in the high dose IC (24-48 $\mu\text{g/kg}$) and IV (18 & 38 $\mu\text{g/kg}$) groups, respectively, was unexpected. The greatest mean increases in ETT of 107.9 and 133.1 seconds at 2 and 6 months, respectively, occurred in the high dose (24-48 $\mu\text{g/kg}$) IC group. The IV group exhibited significant mean increases in ETT of 93.4 seconds and 87.5 seconds, at 2 months and 6 months respectively, which was not 10 predicted by the rat and pig animal models used herein. Overall, the persistence of the effect (increase in ETT) at six months and its magnitude for both the IC and IV groups was wholly unexpected.

The 66 human patients of the Phase I clinical trial described in Examples 1-3 herein were also evaluated using the Seattle Angina Questionnaire 15 (SAQ). The SAQ is a validated, disease-specific, quality of life instrument which assesses the following five scales: 1) "exertional capacity" = limitation of physical activity; 2) "disease perception" = worry about MI; 3) "treatment satisfaction"; 4) "angina frequency" = number of episodes and sublingual nitroglycerin usage; and 5) "angina stability" = number of episodes with most 20 strenuous physical activity. The possible range of scores for each of the five scales is 0 to 100 with the higher scores indicating a better quality of life. Typically, a mean change of 8 points or more between the mean baseline scores (*i.e.*, before treatment) and the post-treatment scores is recognized as being "clinically significant." However, in the present analysis, a dose was considered 25 "effective" if the mean change in score from baseline increased by greater than 14 points. The reason that 14 was chosen (instead of 8) was to allow for the improvement that was seen in the placebo group at 2 months in a clinical trial of another growth factor--VEGF.

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In performing the SAQ evaluation, the patients were categorized according to the same dosage groups that were evaluated for ETT, *i.e.*, 0.33 - 2.0 µg/kg IC (low) 6.0 - 12.0 µg/kg IC (mid); 24 - 48 µg/kg IC (high); and 18 and 36 µg/kg IV. The questionnaire was administered to subjects in each dosage group at baseline (prior to dosing), and at 2 months and 6 months after being administered a single unit dose composition of rFGF-2 in accordance with the method of the present invention.

The first SAQ scale is "exertional capacity." The data on exertional capacity is summarized in Table 2 herein. As reflected in Table 2,

Table 2. Exertional Capacity (EC) - Change from Baseline

<i>FGF-2 Dose Group</i>	<i>Change from Baseline at Two Months</i>	<i>Change from Baseline at Six Months</i>
0.33 to 2.0 µg/kg IC (N = 16)	N = 14 15.0* (-25 to 53)	N = 7 23.2* (0 to 53)
6.0 and 12 µg/kg IC (N = 8)	N = 7 20.2* (-14 to 44)	N = 6 24.1 (-11 to 69)
24.0 to 48.0 µg/kg IC (N = 28)	N = 26 14.6* (-33 to 75)	N = 23 22.9* (-14 to 75)
18.0 and 36.0 µg/kg IV (N = 14)	N = 12 10.9 (-8 to 67)	N = 14 16.5* (-19 to 63)

N = number of subjects; mean (range); * = p < 0.05

the change from baseline in mean score increased at 2 and 6 months for each of the three IC dosage groups and at 6 months for all dosage groups (IC and IV). All scores at all dosage levels increased with time in going from 2 months to 6 months with the best increases (23.2, 24.1, 22.9 and 16.5) relative to baseline being seen at 6 months post-dosing.

The second SAQ scale to be evaluated was "angina stability."

The data summarizing the angina stability is presented in Table 3 herein.

Table 3. Angina Stability (AS) – Change from Baseline

<i>FGF-2 Dose Group</i>	<i>Change from Baseline at Two Months</i>	<i>Change from Baseline at Six Months</i>
0.33 to 2.0 µg/kg IC (N = 16)	N = 13 46.2* (0 to 100)	N = 7 21.4* (0 to 50)
6.0 and 12 µg/kg IC (N = 8)	N = 7 32.1* (0 to 50)	N = 6 16.7 (-25 to 50)
24.0 to 48.0 µg/kg IC (N = 28)	N = 27 34.3* (-25 to 75)	N = 24 17.7* (-25 to 75)
18.0 and 36.0 µg/kg IV (N = 14)	N = 12 39.6* (0 to 100)	N = 14 23.2* (0 to 75)

N = number of subjects; mean (range); * = $p < 0.05$

According to Table 3, the change in score for angina stability increased relative to baseline at both 2 and 6 months for each group. The improvements in angina stability seen at 2 months after dosing (46.2, 32.1, 34.3 and 39.6) were significantly greater than those scores seen at 6 months (21.4, 16.7, 17.7 and 23.2). However, the scores found at both 2 months and 6 months after dosing showed that all dosages were found to be effective (> 14) in increasing angina stability. Moreover, the magnitude of the increases and their duration for 6 months were unexpected.

The third SAQ scale to be evaluated was “angina frequency.” The data summarizing the angina frequency is presented in Table 4 herein.

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Table 4. Angina Frequency (AF) – Change from Baseline

<i>FGF-2 Dose Group</i>	<i>Change from Baseline at Two Months</i>	<i>Change from Baseline at Six Months</i>
0.33 to 2.0 µg/kg IC (N = 16) (low)	N = 14 27.9* (-10 to 80)	N = 7 12.9 (-40 to 50)
6.0 and 12 µg/kg IC (N = 8) (mid)	N = 7 32.9* (0 to 80)	N = 6 36.7 (-10 to 90)
24.0 to 48.0 µg/kg IC (N = 28) (high)	N = 27 28.9* (-40 to 80)	N = 24 25.8* (-30 to 80)
18.0 and 36.0 µg/kg IV (N = 14)	N = 12 20.0* (0 to 90)	N = 14 11.4 (-30 to 60)
ALL GROUPS (N = 66)	N = 60 27.3	N = 51 21.4

N = number of subjects; mean (range); * = $p < 0.05$

According to Table 4, the mean patient scores (27.9, 32.9, 28.9 and 20.0) for angina frequency increased at 2 months (relative to baseline) by an effective amount (> 14) for all dosage groups and for all modes of administration (IC or IV). The mean patient scores continued to increase at 6 months only for the mid dose (6.0 - 12.0 µg/kg) group, suggesting a peak effect at 2 months post-dosing. However, for the mid dose (6.0 - 12.0 µg/kg) and high dose (24.0 - 48.0 µg/kg) groups, the changes at 2 months and 6 months were similar, suggesting a persistent effect at 6 months on angina frequency. The third SAQ scale to be evaluated was "angina frequency." The data summarizing the angina frequency is presented in Table 4 herein.

The fourth SAQ scale to be evaluated was "angina frequency." The data summarizing the angina frequency is presented in Table 5 herein.

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Table 5. Treatment Satisfaction (TS) – Change from Baseline

<i>FGF-2 Dose Group</i>	<i>Change from Baseline at Two Months</i>	<i>Change from Baseline at Six Months</i>
0.33 to 2.0 µg/kg IC (N = 16) (low)	N = 14 8.5* (-19 to 31)	N = 7 6.3 (-25 to 25)
6.0 and 12 µg/kg IC (N = 8) (mid)	N = 7 17.9 (-13 to 44)	N = 6 19.8 (0 to 63)
24.0 to 48.0 µg/kg IC (N = 28) (high)	N = 27 18.8* (-38 to 69)	N = 24 13.0 (-75 to 63)
18.0 and 36.0 µg/kg IV (N = 14)	N = 12 19.8* (-13 to 63)	N = 14 13.4* (-19 to 56)

N = number of subjects; mean (range); * = p < 0.05

According to Table 5, the score for treatment satisfaction increased by an effective amount at 2 months for the mid and high dose IC groups as well as the IV group. At six months post-dosing, only the score for the mid dose group IC had a score that was greater than 14, suggesting a peak effect for treatment satisfaction at 2 months.

The fifth SAQ scale to be evaluated was "disease perception." The data summarizing the disease perception is presented in Table 6 herein. According to Table 6, the scores for disease perception increased from baseline to scores of 20.2-29.2 at 2 months and 23.8-34.0 at 6 months. These scores showed that administering a single unit dose composition in accordance with the method of the present invention was considered to be as effective (or more effective) at 6 months as at two months. These scores suggest a persistence of the effectiveness of the method of the present invention on disease perception out to six months following administration of a single unit dose composition.

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Table 6. Disease Perception (DP) – Change from Baseline

<i>Dose Group</i>	<i>Change from Baseline at Two Months</i>	<i>Change from Baseline at Six Months</i>
0.33 to 2.0 µg/kg IC (N = 16) (low)	N = 14 29.2* (-8 to 58)	N = 7 26.2* (0 to 42)
6.0 and 12 µg/kg IC (N = 8) (mid)	N = 7 20.2* (-8 to 50)	N = 6 30.6* (0 to 58)
24.0 to 48.0 µg/kg IC (N = 28) (high)	N = 27 27.8* (-33 to 92)	N = 24 34.0* (-33 to 100)
18.0 and 36.0 µg/kg IV (N = 14)	N = 12 22.9* (-8 to 92)	N = 14 23.8* (-8 to 75)

N = number of subjects; mean (range); * = $p < 0.05$

- Up to 60 of the human patients of the Phase I clinical trial
- 5 described in Examples 1-3 herein were also evaluated using resting magnetic resonance imaging (MRI) scans of their heart. The resting MRI scans were performed on the patients at baseline, and at 1 month, 2 months and 6 months after dosing with a single unit dose composition of the present invention. The doses were considered “effective” based upon statistical significance ($p < 0.05$).
- 10 The objective criteria assessed by the resting MRI scans are the following: (1) ejection fraction; (2) myocardial infarct extent (%); (3) normal wall thickening (4) normal wall motion (%); (5) target wall thickening (%); (6) target wall motion (%); (7) target wall area collateral extent (%); and (8) target area delayed arrival extent (%).
- 15 Based upon the resting MRI, no change in “ejection fraction” was observed at one month for any one group. The mean change from baseline for all groups ($n = 33$) at 1 month was an increase of 2.0% ($p = 0.042$). At two months, the mean change from baseline for the low dose IC group ($n = 13$) was an increase of 8.1% ($p = 0.007$); and for all groups ($n = 54$), the mean change
- 20 from baseline was an increase of 3.8% ($p = 0.001$). At six months, the mean

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change from baseline for the high dose IC group (n=19) was 5.3% (p=0.023); for the IV group (n=3) was 11.1% (p=0.087); and for all groups (n=33) was 5.7% (p=0.001).

Based upon the resting MRI, there was no statistically significant change in the "myocardial infarct extent" (%) for any group, or for all groups in combination at 1 month, 2 months or 6 months post-dosing. When the normal wall motion (%) and normal wall thickening were assessed, there was no statistically significant change from baseline at 1 month, 2 months or 6 months for any one group. However, there was a statistically significant change from baseline in target wall motion for all groups at one (n=60), two (n=54) and six (n=33) months, which was reflected as a mean increase from baseline of 2.7% (p=0.015), 4.4% (p=<0.001) and 6.4% (p<0.001), respectively. However, there was also a statistically significant change from baseline in target wall thickening for all groups at one (n=60), two (n=54) and six (n=33) months, which was reflected as a mean increase from baseline of 4.4% (p=0.015), 6.3% (p=<0.001) and 7.7% (p<0.001), respectively.

The next criteria assessed by MRI was "target area collateral extent" (%). The mean increase from baseline in target area collateral extent for all groups was highly statistically significant at one month (n=31), two months (n=27) and six months (n=16), wherein the increases were 8.3% (p<0.001), 10.9% (p<0.001) and 11.2% (p<0.001), respectively. The greatest collateral extent increases were observed for the low and mid IC doses, *i.e.*, at one month (10.4% and 18.3%, respectively), two months (14.7% and 18.0%, respectively) and six months (16.0% and no value for mid dose, respectively), which was wholly unexpected. At one month, two months and six months post-dosing, the corresponding % increases in target area collateral extent that were observed for the IC high dose group were 6.3%, 8.0% and 9.0%, respectively.

The final criteria assessed by MRI was "target area delayed arrival extent" (%). The mean decrease from baseline in target area delayed arrival

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extent for all groups was highly statistically significant at 1 month (n=60), 2 months (n=54) and 6 months (n=34), wherein the decreases were -5.8% (p<0.001), -8.3% (p<0.001) and -10.0% (p<0.001), respectively. The greatest target area delayed extent decreases were observed for the low dose IC group, which was also highly unexpected.

Thus, providing CAD patients with a single IC or IV infusion of rFGF-2 in accordance with the present invention provided the patients with a statistically significant physical improvement as objectively measured by MRI and other conventional criteria.

Pharmacokinetics and Metabolism

The molecular structure of FGF-2 contains a positively charged tail that is known to bind to proteoglycan chains (heparin and heparin-like structures) on cell surfaces and on the endothelial wall of the vasculature. See Moscatelli, *et al.*, "Interaction of Basic Fibroblast Growth Factor with Extracellular Matrix and Receptors," Ann. NY Acad. Sci., 638:177-181 (1981).

The kidneys and liver are the major organs for the elimination of rFGF-2. In particular, the kidneys have a protein cutoff of about 60 kD and thus retain serum albumin (MW 60 kD). However, FGF-2 (146 residues) has a molecular weight of about 16.5 kD. Accordingly, renal excretion is to be expected. In a radiolabelled biodistribution study of commercially available bovine FGF-2 (bFGF-2), both the liver and the kidney were shown to contain high counts of the radiolabelled bFGF-2 at 1 hour after IV or IC injection. In a published study, wherein another recombinant iodinated form of bFGF-2 was given to rats, the liver was identified as the major organ of elimination. Whalen *et al.*, "The Fate of Intravenously Administered bFGF and the Effect of Heparin," Growth Factors, 1:157-164 (1989). It is also known that FGF-2 binds in the general circulation to α_2 -macroglobulin and that this complex is internalized by receptors on the Kupffer cells. Whalen *et al.* (1989) and

LaMarre *et al.*, "Cytokine Binding and Clearance Properties of Proteinase-Activated Alpha-2-Macroglobulins," Lab. Invest., 65:3-14 (1991). Labelled FGF-2 fragments were not found in the plasma, but they were found in the urine and corresponded in size to intracellular breakdown products.

5 In preclinical testing, we determined the pharmacokinetics of rFGF-2 (SEQ ID NO: 2) after intravenous (IV) and intracoronary (IC) administration in domestic Yorkshire pigs, and after IV administration dosing in Sprague Dawley ("SD") rats. The pig models demonstrated linear pharmacokinetics (0.65 µg/kg - 20 µg/kg) IC and IV. The terminal half-life of
10 the FGF-2 in the pig model was 3-4 hours. The rat models demonstrated linear pharmacokinetics over the range of 30-300 µg/kg IV. The terminal half-life of the FGF-2 in the rat model was 1 hour. Both species showed plasma concentration suggesting a two-compartment model.

Likewise, in humans, the FGF-2 plasma concentrations after IV and/or IC
15 infusion followed a biexponential curve with an initial steep slope and considerable decrease over several log scales (the distribution phase) during the first hour, followed by a more moderate decline (the elimination phase). Fig. 1A provides a plasma concentration versus time curve showing these phases in humans after IC administration of rFGF-2 of SEQ ID NO: 2 as a function of
20 each of the following eight doses: 0.33 µg/kg, 0.65 µg/kg, 2 µg/kg, 6 µg/kg, 12 µg/kg, 24 µg/kg, 36 µg/kg, and 48 µg/kg of lean body mass (LBM). Figure 1A shows the plasma dose linearity for the eight doses of rFGF-2 that were administered by IC infusion over a twenty minute period. Figure 1A also shows a biphasic plasma level decline, *i.e.*, a fast distribution phase during the first
25 hour, followed by an elimination phase with an estimated $T_{1/2}$ of 5-7 hours. The plasma concentrations of FGF-2 of SEQ ID NO: 2 were determined by a commercially available ELISA (R&D Systems, Minneapolis MN) that was marketed for analysis of human FGF-2. The ELISA assay showed 100% cross-reactivity with the rFGF-2 of SEQ ID NO: 2. Other members of the FGF

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family, as well as many other cytokines, were not detected by this assay. Further, heparin does not interfere with the assay.

Figure 1B is a plot of the mean FGF-2 plasma concentration as a function of time for 18 $\mu\text{g/kg}$ and 36 $\mu\text{g/kg}$ rFGF-2 administered IV, as compared to 36 $\mu\text{g/kg}$ rFGF-2 administered IC. The plasma concentration versus time profiles in Figure 1B for the 36 $\mu\text{g/kg}$ doses by the IV and IC routes are superimposable. However, a first-pass effect with the IC route is not eliminated.

Figure 2 is a plot of mean FGF-2 area under the curve (AUC) in $\text{pg}\cdot\text{min/ml}$ corresponding to Figures 1A and 1B. Figure 2 shows the dose linearity of systemic rFGF-2 exposure (AUC) following IC or IV infusion. In particular, Figure 2 shows that the systemic exposure to rFGF-2 following administration by the IC and IV routes is substantially similar.

Figure 3 is a plot of individual human patient plasma clearance (CL) values (ml/min/kg) as a function of the time of heparin administration in "minutes prior to rFGF-2 infusion." Figure 3 shows the influence of timing of heparin administration on FGF-2 plasma clearance (CL). Although Figure 3 shows that administering heparin up to 100 minutes prior to rFGF-2 decreases FGF-2 clearance, the preferred time for administering heparin is from 0-30 minutes prior the rFGF-2 administration, wherein the effect of the heparin on decreasing FGF-2 clearance is greatest.

Figure 4 is a plot individual human patient rFGF-2 dose normalized area under curves (AUCs) as a function of the time of heparin administration in "minutes prior to rFGF-2 infusion" and shows the influence of timing of heparin administration on rFGF-2 AUC. Figure 4 shows that the greatest AUC/dose was achieved when an effective amount of a glycosoaminoglycan, such as heparin, was preadministered within 30 minutes or less of IC rFGF-2 infusion, more preferably within 20 minutes or less of IC or IV rFGF-2 infusion. Typically, an effective amount of a glycosoaminoglycan is 10-80 U/kg heparin.

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The mean pharmacokinetic parameters for rFGF-2 in humans as a function of dosage and mode of administration are summarized in Table 8 herein. Referring to Table 8, the $T_{1/2}$ for FGF-2 in humans was determined to range from 2.2 ± 3.7 hours at low dose ($0.33\text{-}2.0 \mu\text{g/kg}$) IC to 7.0 ± 3.5 hours at a dose of $18\text{-}36 \mu\text{g/kg}$ IV; given the limitations of the assay, the terminal half-life is estimated at 5-7 hours for all groups. The clearances of FGF-2 ranged from 13.2 to 18.2 L/hour/70kg man. Finally, the steady state volume (V_{ss}) was determined to range from 11.3 ± 10.4 L/70kg man to 16.8 ± 10.7 L/70kg man.

Table 8. Mean rFGF-2 PK Parameters in Humans

FGF-2 Dose $\mu\text{g/kg}$	N	Route	CL (L/hr/70kg)	$t_{1/2}$ (h)	V_{ss} (L/70kg)
0.3 - 2	16	IC	18.2 ± 13.4	2.2 ± 3.7	11.3 ± 10.4
6 - 12	8	IC	13.2 ± 7.3	3.1 ± 2.5	12.1 ± 4.9
24 - 48	28	IC	14.7 ± 8.3	6.3 ± 1.8	16.8 ± 10.7
18 - 36	14	IV	13.9 ± 7.9	7.0 ± 3.5	16.4 ± 8.6

Although the binding of FGF-2 to heparin-like structures is strong (dissociation constant $\sim 2 \times 10^{-9}$ M), the binding of FGF-2 to a specific tyrosine kinase receptor is approximately two orders of magnitude higher (dissociation constant $\sim 2 \times 10^{-11}$ M). Moscatelli *et al.*, (1991). Thus, without being bound to any theory, the complexation of rFGF-2 with a glycosaminoglycan, such as a heparin, might increase signal transduction and mitogenesis, and/or protect the rFGF-2 from enzymatic degradation.

The examples, which follow, provide more details on the selection criterion and the Phase I clinical trial that gave rise to the data discussed above.

EXAMPLE 1

"Unit Dose of rFGF-2 Employed in the Phase I Clinical Trial"

5 The rFGF-2 of SEQ ID NO: 2 was formulated as a unit dose and pharmaceutical composition and administered to rats, pigs and ultimately to humans in the Phase I clinical trial referenced herein. The various formulations are described below.

10 **The rFGF-2 Unit Dose** was provided as a liquid in 3 cc type I glass vials with a laminated gray butyl rubber stopper and red flip-off overseal. The rFGF-2 unit dose contained 1.2 ml of 0.3 mg/ml rFGF-2 of SEQ ID NO: 2 in 10 mM sodium citrate, 10 mM monothioglycerol, 1 mM disodium dihydrate EDTA (molecular weight 372.2), 135 mM sodium chloride, pH 5.0. Thus, in absolute terms, each
15 vial (and unit dose) contained 0.36 mg rFGF-2. The vials containing the unit dose in liquid form were stored at 2° to 8° C.

The rFGF diluent was supplied in 5 cc type I glass vials with a laminated gray butyl rubber stopper and red flip-off overseal. The rFGF-2 diluent contains 10
20 mM sodium citrate, 10 mM monothioglycerol, 135 mM sodium chloride, pH 5.0. Each vial contained 5.2 ml of rFGF-2 diluent solution that was stored at 2° to 8°C.

The rFGF-2 Pharmaceutical Composition that was infused was prepared by
25 diluting the rFGF-2 unit dose with the rFGF diluent such that the infusion volume is 10-40 ml. In order to keep the EDTA concentration below a preset limit of 100 µg/ml, the total infusion volume was increased to a maximum of 40 ml when proportionately higher absolute amounts of FGF-2 were administered to patients with higher body weights.

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EXAMPLE 2

**“Selection Criteria For Patients With Coronary Artery Disease
For Treatment With rFGF-2”**

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The following selection criteria were applied to Phase I patients with coronary artery disease, whose activities were limited by coronary ischemia despite optimal medical management, and who were not candidates for approved revascularization therapies:

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Inclusion criteria: Subject is eligible if:

- Male or female, greater than or equal to 18 years of age
- Diagnosis of coronary artery disease (CAD)
- 15 • Suboptimal candidates for approved revascularization procedures, *e.g.*, angioplasty, stents, coronary artery bypass graft (CABG) (or refuses those interventions)
 - Able to exercise at least three minutes using a modified Bruce protocol and limited by coronary ischemia
 - 20 • Inducible and reversible defect of at least 20% myocardium on pharmacologically stressed thallium sestamibi scan
 - CBC, platelets, serum chemistry within clinically acceptable range for required cardiac catheterization
 - Normal INR, or if anticoagulated with Coumadin, INR <2.0
 - 25 • Willing and able to give written informed consent to participate in this study, including all required study procedures and follow-up visits

30

Exclusion criteria: Subject is not eligible if:

- Malignancy: any history of malignancy within past ten years, with the exception of curatively treated basal cell carcinoma.
- Ocular conditions: proliferative retinopathy, severe non-proliferative retinopathy, retinal vein occlusion, Eales' disease, or macular

edema or funduscopy by ophthalmologist: history of intraocular surgery within six months

- Renal function: creatinine clearance below normal range adjusted for age; protein >250 mg or microalbumin >30 mg/24 h urine
- 5
- Class IV heart failure (New York Heart Association)
 - Ejection fraction < 20% by echocardiogram, thallium scan, MRI or gated pooled blood scan (MUGA)
 - Hemodynamically relevant arrhythmias (*e.g.*, ventricular fibrillation, sustained ventricular tachycardia)
- 10
- Severe valvular stenosis (aortic area <1.0 cm², mitral area <1.2 cm²), or severe valvular insufficiency
 - Marked increase in angina or unstable angina within three weeks
 - History of myocardial infarction (MI) within three months
 - History of transient ischemic attack (TIA) or stroke within six
- 15 months
- History of CABG, angioplasty or stent within six months
 - History of treatment with transmyocardial laser revascularization, rFGF-2, or vascular endothelial growth factor (VEGF) within six months
 - Females of child-bearing potential or nursing mothers
- 20
- Any pathological fibrosis, *e.g.*, pulmonary fibrosis, scleroderma
 - Known vascular malformation, *e.g.*, AV malformation, hemangiomas
 - Coexistence of any disease which might interfere with assessment of symptoms of CAD, *e.g.*, pericarditis, costochondritis, esophagitis, systemic
- 25
- vasculitis, sickle cell disease
 - Coexistence of any disease which limits performance of modified Bruce protocol exercise stress test, *e.g.*, paralysis or amputation of a lower extremity, severe arthritis or lower extremities, severe chronic obstructive pulmonary disease (COPD)

- Participation in clinical trials of investigational agents, devices or procedures within thirty days (or scheduled within sixty days of study drug)
 - Known hypersensitivity to rFGF-2 or related compounds
 - Any condition which makes the subject unsuitable for participation
- 5 in this study in the opinion of the investigator, *e.g.*, psychosis, severe mental retardation, inability to communicate with study personnel, drug or alcohol abuse.

EXAMPLE 3

10 **"Phase I Clinical Study on Recombinant FGF-2
(SEQ ID NO: 2) Administered to Humans"**

15 The Phase I CAD trial of this example is an open label, dose escalation study of recombinant fibroblast growth factor-2 (rFGF-2) for safety, tolerability and pharmacokinetics. The study was conducted at two sites: Beth Israel Deaconess Hospital (Harvard) in Boston, MA and Emory University Hospital in Atlanta, GA. Enrollment is complete. Subjects were treated with a single infusion of rFGF-2 on Day 1 and followed for 360 days; follow-up is not yet complete in some subjects.

20 The subject population consists of patients with advanced CAD who are exercise limited by coronary ischemia and are considered suboptimal candidates for (or do not want to undergo) one of the established revascularization procedures (*e.g.*, CABG, angioplasty -- with or without stent).

25 The major exclusion criteria were history or suspicion of malignancy, uncompensated heart failure or left ventricular ejection fraction <20%, renal insufficiency or proteinuria, and various ocular conditions (*e.g.*, proliferative diabetic retinopathy, severe non-proliferative retinopathy).

30 Sixty-six subjects have received rFGF-2 of SEQ ID NO: 2 in this trial: fifty-two received the rFGF-2 as an IC infusion and fourteen received it as an IV infusion. Each subject was observed in the hospital for at least twenty-four

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hours, and then followed as an outpatient for 360 days with follow-up visits (Days 15, 29, 57, 180 and 360). At least four subjects were studied at each dose; if no subject experienced dose-limiting toxicity as defined by the protocol within six days, the dose was escalated. The drug was administered as a single
5 20 minute infusion divided between two major sources of coronary blood supply (IC), using standard techniques for positioning a catheter into the patient's coronary artery (such as already employed in angioplasty) or in a peripheral vein (IV). The doses in $\mu\text{g/kg}$ of rFGF-2 administered IC (and the number of patients) were 0.33 (n=4), 0.65 (n=4), 2.0 (n=8), 6.0 (n=4), 12.0 (n=4), 24
10 (n=8), 36 (n=10) and 48 (n=10) of rFGF-2 of SEQ ID NO: 2. The doses in $\mu\text{g/kg}$ of rFGF-2 administered IV (and the number of patients) were 18.0 (n=4) and 36.0 (n=10) or rFGF-2 of SEQ ID NO: 2.

Angina frequency and quality of life was assessed by the Seattle Angina Questionnaire (SAQ) at a baseline (before rFGF-2 administration) and at 2
15 months and 6 months after rFGF-2 administration. Exercise tolerance time (ETT) was assessed by the treadmill test at 1, 2, and 6 months. Rest/exercise nuclear perfusion and gated sestamibi-determined rest ejection fraction (EF), and resting magnetic resonance imaging (MRI) were assessed at baseline, and at 1 month, 2 months and 6 months post rFGF-2 administration. MRI measurements which
20 were thought to objectively measure changes in % in cardiac function and perfusion included: (1) ejection fraction; (2) myocardial infarct extent (%); (3) normal wall thickening (4) normal motion (%); (5) target wall thickening (%); (6) target wall motion (%); (7) target wall area collateral extent (%); and (8) target area delayed arrival extent (%).

25 If one of four subjects experienced dose-limiting toxicity (as defined by the protocol), four additional subjects were studied at that dose; if none experienced toxicity, the dose was escalated and another group was studied. The maximally tolerated dose (MTD) was defined as the IC dose which was tolerated by 9/10 subjects, *i.e.*, 36 $\mu\text{g/kg}$ IC.

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Careful fluid management pre-infusion was prescribed using a Swan-Ganz catheter and vital signs were monitored frequently during dosing. Heparin was administered IV prior to the infusion of rFGF-2 in all groups. The EDTA concentration was less than 100 µg/ml in the unit dose composition.

- 5 Volume of study drug administered varied with dose and subject's weight, and ranged from 10 ml at lower doses to 40 ml at higher doses.

Preliminary Results

- 10 The results presented here are unaudited and are based on a third interim analysis for sixty-six subjects with six months follow - up for all groups (1-10) and the serious adverse events (SAE) report of 29 July 1999 from Chiron Drug Safety. Data collection for the last visit (Day 360) and final analysis is in progress.

- 15 The starting dose of 0.33 µg/kg IC was escalated over eight sequential groups to 48 µg/kg IC, at which dose 2 of ten subjects experienced dose-limiting toxicity (hypotension) as defined by the protocol. Hypotension was manageable with fluids alone in all subjects (no vasopressors or assistive devices). At 36 µg/kg IC, only 1 of 10 subjects had dose-limiting toxicity which
20 defined this dose as the maximally tolerated dose (MTD). Two additional groups were studied by IV infusion; four subjects of half the MTD (18 µg/kg) and ten subjects at the MTD (36 µg/kg).

- Hypotension was dose-limiting in humans, as predicted by the animal model in Yorkshire pigs. However, 36.0 µg/kg rFGF-2 IC was tolerated
25 in humans; whereas in pigs, 20.0 µg/kg rFGF-2 IC caused profound hypotension in one of two animals. Better tolerability in humans was attributed to aggressive fluid management and absence of general anesthesia.

- As of 29 July 1999, thirty-three serious adverse events (SAEs) have occurred in 24/66 subjects, but were not dose-related. Fifteen (15) SAEs
30 were considered at least possibly related to rFGF-2; whenever there was a

difference between relatedness assigned by the investigator and the medical monitor, the more conservative relationship was assigned. SAE's were multiple in five subjects: 01103 (0.33 µg/kg IC), 01106 (0.65 µg/kg IC), 01113 (2.0µg/kg IC), 01137 (36.0 µg/kg IV), 02101 (0.65 µg/kg IC).

5 The most frequent treatment-emergent adverse events (AEs) on Day 1 were transient systolic hypotension and transient bradycardia. The hypotension was dose-dependent and occurred more frequently at doses greater than or equal to (\geq) 24 µg/kg IC; bradycardia was not dose-dependent. Other adverse events (AEs) which were deemed at least possibly related and appeared
10 dose-related occurred within the first several days or week post infusion and included chest pain, shortness of breath, insomnia, anxiety, and nausea. These events were mild to moderate in severity, and most did not require specific intervention.

15 When administered IC, the drug was administered over approximately 20 minutes as a single infusion divided between two major sources of coronary blood supply (IC), using standard techniques for positioning a catheter into the patient's coronary artery (such as already employed in angioplasty). When administered IV, the drug was administered as an infusion over 20 minutes into a peripheral vein.

20 The preliminary safety results indicate that serious events were not dose related. Thus far, of the eight IC dosage groups, there were three deaths in the lower dosage groups, *i.e.*, at 0.65 µg/kg (Day 23), at 2.0 µg/kg (Day 57) and at 6.0 µg/kg (Day 63), and one death in the highest dose group, *i.e.*, at 48.0 µg/kg (approximately 4 months post-dosing). Three of the deaths were cardiac;
25 one death was due to a large B cell lymphoma that was diagnosed three weeks after dosing in the patient in group 4 (6.0 µg/kg) who patient died at two months post-dosing.

Acute myocardial infarction (MI) occurred in four patients, *i.e.*, one patient from each of groups 1 (0.33 µg/kg), 3 (2.0 µg/kg), 4 (6.0 µg/kg) and 7

(36.0 µg/kg). Multiple MIs occurred in two patients, *i.e.*, one from group 1 (0.33 µg/kg) and one from group 3 (2.0 µg/kg). Emergency revascularization procedures (CABG or angioplasty with or without stent) were performed during follow-up in 4 patients: one each from groups 1 (0.33 µg/kg), 3 (2.0 µg/kg), 4 (6.0 µg/kg), and 7 (36.0 µg/kg).

Acute hypotension, seen at higher doses during or just subsequent to infusion, was managed by administration of IV fluids without need for a vasopressor. The maximally tolerated dose (MTD) in humans was defined as 36 µg/kg IC. (In contrast, in pigs, the MTD was 6.5 µg/kg IC.) Doses of rFGF-2 up to 48 µg/kg IC were administered in human patients with aggressive fluid management, but were defined by the protocol as "not tolerated" due to acute and/or orthostatic hypotension in two out of ten patients. The terminal half-life of the infused rFGF-2 was estimated at 5 to 7 hours.

The human patients in this study that were treated with a single IC or IV infusion of rFGF-2 of SEQ ID NO: 2 exhibited a mean increase in ETT of 1.5 to 2 minutes. *See* Table 1. This is especially significant because an increase in ETT of greater than (>) 30 seconds is considered significant and a benchmark for evaluating alternative therapies, such as angioplasty. The angina frequency and quality of life, as measured by SAQ, showed a significant improvement at 2 months in all five subscales for the 66 patients (n=66) tested. *See* Tables 26. In Tables 2-6, a mean change of 14 or more was considered "clinically significant."

When 33 human CAD patients were assessed by resting cardiac magnetic resonance imaging (MRI) at baseline, and at 1, 2, and 6 months after receiving a single unit dose composition of the present invention by IC or IV routes, a highly statistically significant increase was observed in target wall thickening, target wall motion and target area collateral extent; a highly statistically significant decrease was observed in target area delayed arrival extent; and no statistically significant changes were observed in normal wall motion, normal wall thickening or myocardial infarct extent.

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In addition to the above criterion (*i.e.*, ETT SAQ, MRI), a treatment is considered very successful if the angiogenic effects last at least six months. In the present Phase I study, the unexpectedly superior angiogenic effects were observed to last up to 6 months in some patients in all dosage groups. Based upon the results already obtained, it is expected that the angiogenic effects may last twelve months or more but do last at least six months in the patients, at which time the procedure could be repeated, if necessary.

EXAMPLE 4

“Proposed Phase II Clinical Study On rFGF-2 (SEQ ID NO: 2) Administered to Humans to Treat Coronary Artery Disease”

The Phase II clinical trial of rFGF-2 for treating human patients for coronary artery disease is performed as a double blind/placebo controlled study having four arms: placebo, 0.3 µg/kg, 3.0 µg/kg, and 30 µg/kg administered once IC. This study is ongoing and results are not yet available.

EXAMPLE 5

“Unit Dose and Pharmaceutical Composition of rFGF-2 for the Phase II Human Clinical Trial”

The rFGF-2 of SEQ ID NO: 2 was formulated as a unit dose pharmaceutical composition for administration to humans in the Phase II clinical trial referenced herein. The various formulations are described below.

The rFGF-2 Unit Dose was prepared as a liquid in 5 cc type I glass vials with a laminated gray butyl rubber stopper and red flip-off overseal. The rFGF-2 formulation contains 0.3 mg/ml rFGF-2 of SEQ ID NO: 2 in 10 mM sodium citrate, 10 mM monothioglycerol, 0.3 mM disodium dihydrate EDTA (molecular weight 372.2), 135 mM sodium chloride, pH 5.0. Each vial contained 3.7 ml of rFGF-2 drug product solution (1.11 mg rFGF-2 per vial). The resulting unit dose in liquid form is stored at less than -60° C. The above described unit dose

is diluted with the "rFGF-2 placebo." Depending on the size of the patient, the contents of several of the vials may be combined to produce a unit dose of 36 µg/kg for the Phase II study.

- 5 **The rFGF-2 placebo** is supplied as a clear colorless liquid in 5 cc type I glass vials with a laminated gray butyl rubber stopper and red flip-off overseal. The rFGF-2 placebo is indistinguishable in appearance from the drug product and has the following formulation: 10 mM sodium citrate, 10 mM monothioglycerol, 0.3 mM disodium dihydrate EDTA (molecular weight 372.2), 135 mM sodium chloride, pH 5.0. Each vial contains 5.2 ml of rFGF-2 placebo solution. Unlike
10 the unit dose, the rFGF-2 placebo is stored at 2° to 8°C.

- 15 **The rFGF-2 Pharmaceutical Composition** that is infused is prepared by diluting the rFGF-2 unit dose with the rFGF diluent such that the infusion volume is 20 ml for Phase II.

SEQUENCE LISTING

<110> Whitehouse, Martha J.

<120> Angiogenically Effective Unit Dose of FGF-2 and Method of Use

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